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Evaluation of Chemicals Used for Drinking Water Disinfection for Production of Chromosomal Damage and Sperm-Head Abnormalities in Mice

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Chemical oxidants are commonly added during water treatment for disinfection purposes. These chemicals have not been tested previously for their ability to induce genetic damage in vivo. Chlorine (hypochlorite and hypochlorous acid), monochloramine, chlorine dioxide, sodium chlorite, and sodium chlorate were evaluated for induction of chromosomal aberrations and micronuclei in bone marrow of CD-1 mice, and for induction of sperm-head abnormalities in B6C3F1 mice. Oral administration of chlorine at pH 8.5 (where hypochlorite predominates) at dose levels equivalent to approximately 4 and 8 mg/kg/day induced significant increases in the level of sperm-head abnormalities. There was no evidence of other effects with any of the disinfectants. Halogenated acetonitriles, which have previously been shown to form in the stomach following oral dosing of sodium hypochlorite to rats, were also tested in the sperm-head abnormality assay but gave no indication of an effect.

Key words: water treatment, disinfectants, chromosomal aberrations, micronuclei, sperm abnormalities, haloacetonitriles

INTRODUCTION

Disinfectant chemicals are generally added during drinking water treatment in levels sufficient to produce residual concentrations of the disinfectant in the distribution system. One important public health question pertinent to this practice is whether the chronic ingestion of residual levels of disinfectants results in a significant genetic

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or carcinogenic hazard to the human population. Both chlorine (present during water treatment as a mixture of hypochlorous acid and hypochlorite) and monochloramine have been reported to be weakly mutagenic to bacteria [Shih and Lederberg, 1976; Wlodkowski and Rosenkranz, 1975] and to preferentially kill DNA repair deficient strains of bacteria [Shih and Lederberg, 1976; Rosenkranz, 1973], an indication of DNA damage. There is evidence to indicate that these effects are not due to the disinfectants themselves but rather to reaction products. Sodium hypochlorite has been shown to react with amino acids in vitro to form halogenated acetonitriles [Trehy and Bieber, 1981], which possess carcinogenic and mutagenic properties [Bull and Robinson, 1985; Meier et al, 1983]. Genotoxic effects have been observed in bacterial systems following chlorination of amino acids [Süssmuth, 1982]. Recently, Mink et al [1983] have demonstrated the formation of several halogenated compounds, including chloroform, di- and trichloroacetic acid, and di- and trichloroacetonitrile, in the stomach content or blood plasma of rats following oral administration of sodium hypochlorite. The present study was carried out to determine whether three chemicals currently being used for drinking water disinfection (chlorine, chlorine dioxide, and monochloramine) and two reaction products of chlorine dioxide (chlorite and chlorate) induce genotoxic effects following oral administration to mice. For this evaluation mouse bone marrow micronucleus and cytogenetic studies were conducted to determine whether the disinfectants were capable of damaging chromosomes in a somatic cell type. The mouse sperm-head assay was used to test the ability of the disinfectants to disrupt normal sperm morphology as a measure of mutagenic potential to a germ cell line.

MATERIALS AND METHODS

Chemicals

A stock solution of NaOCl (approximately 31 g/liter Cl) was prepared by bubbling Cl_2 into a 1 M solution of NaOH until pH 12 was reached. The concentration of chlorine was determined by iodometric titration (Method 409A) [American Public Health Association, 1975]. Solutions of chlorine for dosing animals were prepared by adjusting the pH with 2.5 N HCl to either 8.5 (predominant chlorine species: OCl^-) or 6.5 (predominant chlorine species: HOCl), and diluting with distilled H_2O to concentrations of 200 mg/liter, 100 mg/liter, and 40 mg/liter in chlorine equivalents. Monochloramine (NH_2Cl) solutions were prepared by slowly adding the NaOCl stock solution to 1.5 M NH_4OH in a molar ratio of 1:1. The Cl concentration was determined as above, and then adjusted to the same concentrations as those used for chlorine dosing. The pH was adjusted to 8.5 by addition of 2.5 N HCl. Chlorine dioxide (ClO_2) solutions were prepared by reacting sodium chlorite ($NaClO_2$) with H_2SO_4, removing free chlorine with a $NaClO_2$ trap, and collecting the generated ClO_2 in distilled H_2O. The concentration of ClO_2 was determined by absorbance at 359 nm (A359 for 1 mM ClO_2 = 1.10). The concentration was adjusted to 400 mg/liter, 200 mg/liter, and 100 mg/liter Cl equivalents in distilled H_2O. Sodium chlorite and sodium chlorate ($NaClO_3$) were obtained commercially as reagent grade chemicals. Solutions were prepared gravimetrically in distilled H_2O at concentrations of 1000 mg/liter, 500 mg/liter, and 200 mg/liter.

Monochloroacetonitrile (CH_2ClCN), trichloroacetonitrile (CCl_3CN), and dibromoacetonitrile ($CHBr_2CN$) were purchased from Aldrich Chemical Company. Dich-

loroacetonitrile ($CHCl_2CN$) was purchased from Pfaltz and Bauer, Inc. The purity of these compounds was found to be greater than 99% by gas chromatographic (GC) analysis. Bromochloroacetonitrile ($CHClBrCN$) was synthesized in our laboratories at a purity of 97.4% bromochloroacetonitrile and 2.5% dichloroacetonitrile.

Animals

Male and female 8–11-week-old Swiss mice of strain CD-1 (Charles River Breeding Laboratories, Inc.) were used for the micronucleus and bone marrow aberration studies. This randomly outbred strain was selected to ensure genetic heterogeneity. For the sperm-head abnormality tests, 8–11-week-old hybrid mice of strain B6C3F1 (Charles River Harlan Industries, Inc.) were selected because of their low and consistent background levels of abnormal sperm. Animals were group housed, separated by sex and by treatment group, and allowed food (Purina Laboratory Chow) and water ad libitum.

Animals were dosed by oral gavage with 1 ml of test solution on a subchronic regimen (five daily administrations approximately 24 hr apart). Concurrent negative controls received deionized water, the diluent of the test solutions. Positive controls were also included to ensure that the assays were working properly. Generally these were also concurrent. The intraperitoneal (IP) route was used for administration of the positive control chemicals since this route was found to be suitable for eliciting consistent, effective responses in all three assays employed.

Micronucleus Assay

Five males and five females were used for each treatment group (three dose levels of the test solution and controls). The positive control was 1 mg/kg triethylenemelamine (TEM) in 0.9% saline, administered IP as split dose concurrently with the last two days of dose administration of the test solutions. Six hours after the last (fifth) dose administration, animals were killed with CO_2 or by cervical dislocation and the tibiae excised. The marrow was flushed from the bone into centrifuge tubes containing 3 ml of fetal calf serum. After centrifugation and resuspension of the marrow, portions of the resultant cell suspension were spread on slides, air-dried, and stained in May-Gruenwald solution and Giemsa. One thousand polychromatic erythrocytes (PCEs) were scored for micronuclei for each animal. The percent micronucleated PCEs per animal was the endpoint used in evaluation of the data. The data from male and female animals were analyzed both separately and combined. A Student's t-test was used to test for differences between each treatment level and the concurrent negative control. A significance level of 0.01 was used to indicate a positive response. This level was chosen to adjust for the multiple t-tests used.

Bone Marrow Aberration Assay

In addition to the subchronic dosing regimen described above an acute administration was also employed in this assay. Eight animals (four males and four females) were used per treatment group. Animals dosed acutely were sacrificed 6, 24, and 48 hr after exposure; animals dosed subchronically were sacrificed 6 hr after the last exposure. The positive control was 1 mg/kg TEM in 0.9% saline administered IP as a one-time (acute) administration concurrently with the 24 hr acute component of the study. Three hours prior to sacrifice, animals were injected IP with 4.0 mg/kg colchicine to collect metaphases. Animals were killed and the marrow obtained in

Hanks' balanced salt solution by procedures similar to those already described for the micronucleus assay. After centrifugation the marrow was treated successively with hypotonic (0.075 M) KCl and with fixative (3:1 methanol:acetic acid). After storage of the fixed material at 4°C for at least overnight, slides were prepared and stained with 5–10% Giemsa at pH 6.8. A mitotic index was determined by scoring the number of cells in mitosis based on at least 500 cells. Fifty metaphase spreads were scored for each animal where possible for structural and numerical aberrations. Numerical aberrations included cells showing either hyperploidy or polyploidy. Structural aberrations included chromosome and chromatid breaks, chromatid deletions, fragments, translocations, triradials, quadriradials, pulverized chromosomes, pulverized cells, complex rearrangements, ring chromosomes, dicentric chromosomes, and minute chromosomes. Four endpoints were evaluated for statistically significant differences between the response at each treatment level and the concurrent negative control, using a Student's t-test at a significance level of 0.01. The endpoints were (1) number of structural aberrations present per animal, (2) number of numerical aberrations present per animal, (3) percentage of cells with at least one structural aberration present per animal, and (4) percentage of cells with two or more structural aberrations per animal. Data for male and female animals were analyzed both separately and combined.

Sperm-head Abnormality Assay

Ten males were used for each treatment group. At 1, 3, and 5 weeks after the last (fifth) dose administration (using the subchronic dosing regimen described above), animals were sacrificed with CO_2 or by cervical dislocation. The positive control was ethyl methanesulfonate (EMS) in deionized water at 200 mg/kg, administered IP in five daily doses. A positive control was used for each kill time. The caudae epididymides were dissected and placed into a petri dish containing 0.9% saline. After dicing with a scissors, the suspension was gently pipetted five to six times in and out of a 5- or 10-ml pipette. The suspension was strained through a 80-μm silk mesh to remove tissue fragments, and 0.5 ml of the filtrate was stained in a centrifuge tube with 0.05 ml 1% Eosin Y. Slides were prepared from this suspension by spreading a drop over the slide with three passes of the edge of another slide. One thousand sperm-heads (500 by each of two readers) were scored per animal, where possible, for sperm-head shape abnormalities using the categories of Wyrobek and Bruce [1975.]. Either an analysis of variance procedure or Student's t-test ($\alpha = 0.01$) was used to analyze the data for differences in the percent sperm-head abnormalities per animal between treated animals and concurrent controls. In the case where reader differences were to be examined, a two-way (dose and reader) analysis of variance was done. Outliers were excluded from the analysis, as recommended by Soares et al [1979], using Dixon's Test for Outliers [Dixon and Massey, 1969] on the set of scores for each reader at each dose level. An arcsin transformation of the data was used to stabilize the variance for the analysis of variance. A positive response was based upon a significant increase at any dose level over the concurrent control using a significance level of 0.05.

RESULTS

The results from testing the disinfectants in the mouse micronucleus assay are shown in Table I. OCl^- produced statistically significant elevation at the 0.05 level

TABLE I. Activity of Chemicals Used for Drinking Water Disinfection in the Mouse Micronucleus Assay

Sample	% Micronucleated cells[a]: dose[b]			
	0	1:4	1:1	Undiluted
OCl^-	0.01 ± 0.01	0.04 ± 0.02	0.10 ± 0.03	0.12 ± 0.4
HOCl	0.10 ± 0.02	0.05 ± 0.02	0.06 ± 0.02	0.08 ± 0.03
NH_2Cl	0.08 ± 0.04	0.08 ± 0.04	0.04 ± 0.02	0.11 ± 0.06
ClO_2	0.12 ± 0.04	0.10 ± 0.04	0.04 ± 0.02	0.13 ± 0.03
ClO_2^-	0.10 ± 0.04	0.10 ± 0.04	0.14 ± 0.07	0.20 ± 0.07
ClO_3^-	0.29 ± 0.07	0.18 ± 0.08	0.18 ± 0.06	0.14 ± 0.04

[a]Values expressed as percent micronucleated cells, mean per animal ± the SEM for both sexes combined. Triethylenemelamine (TEM) was used as a positive control twice during the course of this study. Administration of TEM at a dose level of 0.5 mg/kg/day × 2 gave rise to a mean per animal of 2.25 ± 0.40% micronucleated cells, based on a total of 20 animals.

[b]Concentrations of the undiluted samples equal 200 mg/liter for OCl^-, HOCl, and $ClNH_2$; 400 mg/liter for ClO_2; and 1,000 mg/liter for ClO_2^- and ClO_3^-. Dose was 1.0 ml of the indicated solutions by gavage for 5 successive days. Bone marrows were harvested 6 hr after the final exposures.

but not at the 0.01 level in the number of micronucleated polychromatic erythrocytes at the two highest dose levels for the combined data from both sexes. Since significant increases were not seen in either sex considered separately (data not shown) and since the concurrent negative control for this assay was low in comparison with the other controls, the increase in the combined data was not considered biologically significant. None of the other chemicals tested gave significant increases for either pooled or individual sex data. The positive control chemical, triethylenemelamine (TEM), induced a significant number of micronuclei at the 0.01 level of significance for both male and female animals.

Table II shows the results obtained in the mouse bone marrow cytogenetics assay after treatment of animals with the disinfectants using either a subchronic dosing regimen or a single acute dose administration (24-hr sacrifice). There were no significant differences from control for any of the treatment groups in either structural or numerical chromosomal aberrations for any of the four endpoints analyzed. Negative results were also obtained following a single acute dose administration with cytogenetic evaluations of animals at sacrifice times of 6 and 48 hr (data not shown). TEM gave rise to consistent, significant increases in both structural and numerical chromosomal aberrations relative to control.

Data obtained in the sperm-head abnormality assay at 1 and 5 weeks following treatment with the disinfectants indicated no treatment related differences in the percentage of abnormal sperm-heads (data not shown). Table III shows the results which were obtained at 3 weeks post-treatment. The negative control values, which determined with each chemical assayed, varied from 0.91% to 3.33% abnormal sperm. These values were within the range of laboratory historical controls. Several factors are known to contribute to variation in background frequencies of sperm abnormalities including effects on the animals (eg, age and seasonal effects [Grahn and Farrington, 1983]), as well as differences among readers in scoring of slides. Because of differences in time between the testing of different chemicals or retesting of the same chemical, and because of the involvement of different scorers for different experiments (vs the same two scorers within the same experiment), concurrent rather than historical control values were used in the analysis of the data. The factors tested

TABLE II. Activity of Chemicals Used for Drinking Water Disinfection in the Mouse Bone Marrow Cytogenetics Assay

Sample	Dose type[d]	% Cells with chromosomal aberrations[a,b]: dose[c] 0 S	0 N	1:4 S	1:4 N	1:1 S	1:1 N	Undiluted S	Undiluted N
OCl^-	Sub	0.8	1.8	0.0	2.6	0.0	2.8	2.0	1.0
	Ac	0.6	0.9	0.5	2.0	0.5	1.2	3.0	1.2
HOCl	Sub	1.5	2.0	3.0	1.2	0.8	1.9	2.0	1.1
	Ac	0.5	1.8	1.5	2.0	2.0	1.0	1.5	1.2
NH_2Cl	Sub	0.5	0.2	0.0	0.0	0.4	1.3	0.0	2.0
	Ac	0.0	1.0	0.5	1.4	0.5	0.0	0.0	0.3
ClO_2	Sub	0.0	1.5	0.2	0.8	0.5	1.0	0.6	0.0
	Ac	0.9	0.9	0.6	0.6	0.0	0.8	0.8	2.7
ClO_2^-	Sub	0.6	0.9	0.5	0.2	1.4	1.7	0.5	0.0
	Ac	0.3	0.6	0.0	2.0	0.5	3.0	0.5	1.2
ClO_3^-	Sub	0.8	2.0	1.2	1.2	0.2	2.5	1.3	1.6
	Ac	0.9	0.5	1.0	2.0	0.0	0.0	0.8	1.2

[a]Values expressed as the mean percent of cells per animal with structural (S) or numerical (N) aberrations. Eight animals were used per dose group. Data from both sexes were combined. Fifty metaphase cells were scored per animal, if possible. This was true in 93% of the cases.

[b]Triethylenemelamine was administered as a positive control by single IP injection of 1 mg/kg, with animal sacrifice 24 hr later. The mean% structural and numerical abberations form 24 animals were 33.9 and 7.2, respectively.

[c]Concentrations of the undiluted samples equal 200 mg/liter for OCl^-, HOCl, and $ClNH_2$; 400 mg/liter for ClO_2; and 1,000 mg/liter for ClO_2^- and ClO_3^-. Dose was 1.0 ml of the indicated solutions by gavage.

[d]Sub = subchronic dosing (5 daily administrations) with sacrifice at 6 hr following the last dose; Ac = acute dosing with sacrifice 24 hr later.

in the analysis for significant differences were dose, reader and a dose by reader interaction. A significant effect in the dose by reader interaction would imply that the dose response pattern differed between readers. A significant interaction or a significant difference between readers presented potential problems in the interpretation of the results for each of the chemicals tested. If the examination of reader differences and the overall result indicated that a significant assay response may have been masked by the differences between readers, then each reader's data were analyzed separately.

Even though there were significant reader differences, the combined dose scores for OCl^- showed a significant increase over control in sperm-head abnormalities for both the medium and high dose levels. In a repeat experiment with the same dose levels of OCl^- a significant increase over the controls was found at each dose. When considered separately, the scores for three out of the four readers showed a significant increase over the controls in percent of abnormal sperm-heads. The overall dose-response pattern that is evident from both experiments with OCl^- indicates that OCl^- increased the percent sperm-head abnormalities in a dose-related manner at doses equivalent to 1.6 and 4.0 mg/kg body weight per day. The levels of increase vs the concurrent control values were similar in both the initial and repeat experiments, reaching about twice the control values at the 4.0 mg/kg dose. A higher dose of 8.0 mg/kg resulted in no further increases above that seen at the 4.0 mg/kg dose. Based on these findings of a reproducible, dose-related, and statistically significant response, OCl^- was judged to be positive in the assay.

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TABLE III. Activity of Chemicals Used for Drinking Water Disinfection in the Mouse Sperm Head Abnormality Assay

Sample	% Abnormal sperm-head[a]: dose[b]			
	0	1:4	1:1	Undiluted
OCl^-	2.12 ± 0.19	2.81 ± 0.16	4.07 ± 0.39**	3.68 ± 0.47**
OCl^-[c]	0.91 ± 0.09	1.41 ± 0.15**	1.74 ± 0.13**	1.37 ± 0.10*
HOCl	2.73 ± 0.31	2.07 ± 0.19	1.36 ± 0.11	1.43 ± 0.08
HOCl[c]	1.06 ± 0.06	1.24 ± 0.08	1.01 ± 0.10	1.02 ± 0.08
NH_2Cl	1.16 ± 0.19	1.10 ± 0.09	2.57 ± 0.50**	2.39 ± 0.41*
NH_2Cl[c]	2.06 ± 0.23	2.00 ± 0.17	1.66 ± 0.14	1.51 ± 0.17
ClO_2	1.22 ± 0.12	1.51 ± 0.17	1.54 ± 0.14	1.43 ± 0.17
ClO_2^-	3.24 ± 0.22	2.26 ± 0.22	1.96 ± 0.09	2.27 ± 0.20
ClO_3^-	3.33 ± 0.64	2.29 ± 0.30	3.02 ± 0.33	2.92 ± 0.28

[a]Values expressed as mean percent of abnormal sperm-head per animal ± the SEM. Ethyl methanesulfonate was administered as a positive control by IP injection at a dose of 200 mg/kg/day for 5 days. Mean response was 18.36 ± 4.47% of abnormal sperm.

[b]Concentrations of the undiluted samples were 200 mg/liter for OCl^-, HOCl, and $ClNH_2$; 400 mg/liter for ClO_2, and 1,000 mg/liter for ClO_2^-. Dose was 1.0 ml of the indicated solutions by gavage for 5 successive days. Animals were sacrificed 3 weeks after the final exposures.

[c]Retrial of chemical.

*Significantly elevated above control at $p \leq 0.05$.

**Significantly elevated above control at $p \leq 0.01$. Significant differences were determined by repeated measures ANOVA.

HOCl, the protonated form of chlorine in water, failed to produce significant increases in sperm-head abnormalities. This result was somewhat surprising since OCl^- should be converted to HOCl in the acid pH of the stomach. Thus, similar results for the two species of chlorine might be expected. However, a repeat experiment with HOCl confirmed the negative result.

The results with monochloramine were somewhat ambiguous. In the initial assay with this compound significant elevations in the percent abnormal sperm-heads over control were observed for both the medium and high dose levels. When the responses for each reader were examined individually, it could be seen that these elevations were due entirely to the scoring of one reader. In the repeat assay with this compound, there was no indication of a positive response using either reader's scores. Therefore, monochloramine was judged to be negative in this assay.

Chlorine dioxide failed to produce a significant increase in sperm-head abnormalities at doses equivalent to 3.2, 8.0, or 16.0 mg/kg/day. Furthermore, neither chlorite nor chlorate, two inorganic by-products of chlorine dioxide, induced significant increases in the level of sperm-head abnormalities at doses of 8, 20, or 40 mg/kg/day.

Because several halogenated acetonitriles have been shown to be formed in vitro following chlorination of amino acids [Trehy and Bieber, 1981], and in vivo in rat stomach contents [Mink et al., 1983], it seemed possible that the positive results with hypochlorite in the sperm-head abnormality assay might be attributable to one or more of these compounds. Therefore, mono-, di-, and trichloroacetonitrile; bromochloroacetonitrile; and dibromoacetonitrile were tested for their ability to induce sperm-head abnormalities in mice. The highest total dose level for testing these compounds (ie, 250 mg/kg) was chosen to approximate the single oral dose LD_{50}. The LD_{50} values have been determined in mouse for dichloroacetonitrile and dibro-

monoacetonitrile to be 275 mg/kg and 295 mg/kg, respectively (L. Condie, personal communication) and in rat for monochloroacetonitrile and trichloroacetonitrile to be 220 mg/kg and 250 mg/kg, respectively [NIOSH Registry, 1978]. The results obtained at 3 and 5 weeks following administration of these compounds by oral gavage to mice are shown in Table IV. No treatment-related effects were seen with any of these five compounds.

DISCUSSION

The present data indicate that hypochlorite, the form of chlorine that predominates at normal drinking water pH, may represent a mutagenic hazard, based on the results of one in vivo test for mutagenic potential. Observations of increased sperm-head abnormalities in mice at 3 weeks following treatment, but not at 1 week or 5 weeks, suggests that the effect may be specific to late primary spermatocytes, a cell type in spermatogenesis which undergoes meiotic division and which is subject to mutation.

Although the response observed with hypochlorite was weak, the minimum dose at which an effect was seen was relatively low in comparison to other compounds positive in this assay [Wyrobek et al, 1983]. In relation to levels of expected human exposure, the dose levels employed in the present studies were fairly high. Normally in the United States chlorine is applied during drinking water treatment at levels of 5 to 15 mg/liter, which varies depending on the water source. The residual chlorine levels following treatment are generally 1 to 2 mg/liter. Assuming an average drinking water consumption of 2 liters per day by a 70-kg man, the adult human dose on a per kg basis would be approximately 0.03 to 0.06 mg/kg/day. The lowest dose levels applied to the mice were 25 to 50 times higher. However, exposures were limited to 5 days, rather than the lifetime human exposures. Furthermore, considering the one-hit theory of mutation induction, an effect seen at these doses may be significant in terms of a potential human risk.

Pe[illegible] trying to relate the effects seen with hypochlorite to a [illegible]inty of the induction of sperm-head abnormalities as

in the Mouse Sperm-Head Abnormality Assay[a]

		Abnormal sperm-head mean per animal ± SE: dose (mg/kg/day)			
		0	12.5	25	50
		1.09 ± 0.19	0.93 ± 0.09	0.94 ± 0.09	1.01 ± 0.15
		1.04 ± 0.10	1.20 ± 0.12	1.00 ± 0.14	1.18 ± 0.12
		1.29 ± 0.12	1.06 ± 0.07	1.11 ± 0.14	1.23 ± 0.11
[illegible]CN		1.04 ± 0.14	1.16 ± 0.10	1.21 ± 0.23	1.23 ± 0.14
CCl_3CN		0.44 ± 0.06	0.55 ± 0.10	0.38 ± 0.02	0.56 ± 0.13
CCl_3CN	5	0.57 ± 0.07	0.80 ± 0.07	0.67 ± 0.06	0.68 ± 0.19
$CHClBrCN$	3	1.71 ± 0.07	1.47 ± 0.15	1.31 ± 0.25	1.20 ± 0.09
$CHClBrCN$	5	1.21 ± 0.17	1.02 ± 0.14	0.86 ± 0.06	1.18 ± 0.11
$CHBr_2CN$	3	0.62 ± 0.08	0.84 ± 0.10	0.62 ± 0.05	0.53 ± 0.07
$CHBr_2CN$	5	0.67 ± 0.07	0.71 ± 0.07	0.86 ± 0.09	0.54 ± 0.07

[a]Ethylmethanesulfonate was administered as a positive control in five IP injections (24 hr apart) of 200 mg/kg. The mean percent abnormal sperm values ± SE ranged from 2.86 ± 0.47 to 16.03 ± 2.23 for 3 weeks post-administration and 4.13 ± 0.52 to 24.79 ± 3.31 for 5 weeks post-administration.

a genetic endpoint. The genetic basis for the assay has been discussed in detail elsewhere [Wyrobek and Bruce, 1978]. However, the meaning of positive results in the mouse sperm-head abnormality assay is currently the subject of debate, and the assay is still undergoing development and validation. The assay was originally developed by Wyrobek and Bruce [1975] as a method for screening agents with carcinogenic, mutagenic, or teratogenic properties. More recently, it has been proposed that the assay may be useful for specifically identifying mammalian mutagens rather than carcinogens [Topham, 1980]. This notion is supported by a review of the available data in a recent report of the USEPA's Gene-Tox Program [Wyrobek et al, 1983], which shows the assay to be highly sensitive to germ cell mutagens but not to carcinogens. The specificity of the assay for detecting mammalian mutagens has not been found to be good (ie. high number of false positives); but because of the limited data base involved, this finding requires further assessment. Therefore, it seems prudent at the present time to consider the positive test results with hypochlorite in this assay as presumptive evidence for the mutagenic potential of this compound.

Because of their reactivity it is unlikely that substantial levels of unchanged disinfectants could actually reach the testes. In fact, when chlorine (either at pH 6.7 or at pH 8.5) was allowed to react with stomach contents of mice in vitro at levels comparable to those used in the in vivo assays (ie. 1 ml at 100 mg/liter), no free chlorine residual was detectable within 10 sec, the shortest time that could be measured. This result indicates that the positive result obtained with OCl^- in the sperm-head abnormality assay is most likely attributable to the formation of chlorination by-products(s). The difference in results at pH 8.5 vs pH 6.5 indicates that the reaction between OCl^- and stomach contents is rapid compared to its expected conversion to HOCl at the acid stomach pH, and suggests a difference in the types of by-products produced with OCl^- vs HOCl. Recently, Mink et al [1983] have identified a number of halogenated organic compounds that were formed upon the incubation of rats with high doses of chlorine at pH 8.5. Included in the by-products were haloacetonitriles and trihalomethanes; chemicals that possess both carcinogenic and mutagenic properties. It was of interest to know to what extent the sperm-head abnormality induction observed with OCl^- could be accounted for by these compounds. The results from the present study indicate that none of the haloacetonitriles tested exhibit evidence for sperm-head abnormality induction at the doses levels tested (Table IV). Chloroform has been previously found to be negative for induction of sperm-head abnormalities in mice in one study [Topham, 1980]. A second study found statistically significant, elevated levels of sperm-head abnormalities in mice exposed to air concentrations of 0.04 and 0.08 percent of chloroform, 4 hr/day for 5 days [Land et al, 1981]. However, it is unlikely that the doses of OCl^- in the present study could produce sufficient chloroform to match the doses involved in the Land et al [1981] study.

Observation of maxima in the dose-response curves for two experiments with OCl^- suggests a process that limits the response to rather low levels of exposure. A variety of explanations exist for such a dose-response curve. However, if the effects are to be accounted for by reaction products with organic constituents in the gastrointestinal tract, the response would most likely be limited either by the availability of reactant(s) or by the fact that a product(s) formed at low concentrations of disinfectant is destroyed at higher concentrations. In either case, the product(s) formed must be present in low concentrations. This obviously will make identification of these products difficult.

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Failure of any of the disinfectants to induce chromosomal aberrations or increased numbers of micronuclei in the bone marrow of mice would suggest that they lack clastogenic activity. However, it should be pointed out that these experiments were conducted at doses that did not produce any overt toxicity or lethality. The doses possible within the context of these experiments were limited to stable concentrations of the chemicals that could be produced in aqueous solution. Alternative solvents (organic) were not possible because of a high likelihood of reaction with the disinfectants. Several explanations are possible to account for the fact that OCl^- did not show evidence of clastogenic effects in mouse bone marrow but did induce significant increases in abnormal sperm, using similar dosing regimens. The different assay results may reflect differences in assay sensitivities. The work of Bruce and Heddle [1979] from tests of 61 compounds indicates that the sperm morphology assay has a greater success rate than the micronucleus assay for detecting agents that are active in vivo. The differences in assay detection capabilities may result from the fact that sperm-head abnormalities are thought to arise primarily from point mutations rather than from gross chromosomal damage [Wyrobek and Bruce, 1978]. There also may be differences in susceptibility owing to the strain of mice used (B6C3F1 vs CD-1), or in the tissue distribution or metabolic capacities of the bone marrow vs testes that may influence the specificity of these tissues as targets for the disinfectant byproducts. Finally, it should be noted that the detection of a significant response is dependent on the power of the statistical evaluations involved in each assay. This is apparent from examining the minimum difference between treatment and negative control animals which were required for a result to be considered statistically significant. The minimum differences for statistical significance at the 0.01 level ranged from 0.041 to 0.285 for the micronucleus assay results (Table I), 1.5 to 10.4 for the bone marrow cytogenetic assay results (Table II), and 0.24 to 2.605 for the sperm morphology assay results (Tables III, IV).

In summary, it appears that the drinking water disinfectant most widely applied in the United States is capable of producing positive results in one in vivo bioassay for mutagenic chemicals. Further studies are needed to confirm this result in other mutational test systems, especially in view of the indirect nature and uncertainty of sperm abnormality induction as a genetic endpoint. At present it is not possible to project from these data the degree of hazard that may be implied in terms of increased carcinogenic and/or mutagenic risks associated with the use of chlorine. Furthermore, a more precise understanding of products formed by reactions of OCl^- with organic constituents in the gastrointestinal tract is essential to the determination of whether such risks, if confirmed, outweigh the benefits of disinfection (in terms of preventing waterborne infectious disease) or hazards that are associated with alternative disinfectants such as ClO_2 (eg, hemolytic anemia and hypothyroidism).

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